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CONTRIBUTIONS TO A STUDY OF THE RELATIONS OF SOME
TYPES OF *P. PESTIS* IN MIXED CULTURES

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CONTRIBUTIONS TO A STUDY OF THE RELATIONS OF SOME
TYPES OF *P. PESTIS* IN MIXED CULTURES

[Following is the translation of an article by L. N. Klassovskiy and L. I. Terentyeva, published in the Russian-language periodical Materially Nauchnoy Konferentsii po Prirodnoy Ochagovosti i Profilaktike Chumy (Materials from the Scientific Conference on the Natural Focalness and Prophylaxis of Plague), Alma Ata, Feb. 1963, pages 106-108. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

The present work is an attempt to evaluate the nature of displacements, taking place in populations of the plague causative agent, which are made up of cells which are different from each other based on several signs.

In the test we took four variants of plague bacteria:

- 1 a) a sensitive to streptomycin (S-), glycerol-negative (G-), rhamnose-negative (R-) variant of the EV strain (EV-S-G-R).
- 1 b) a highly resistant to streptomycin variant of the EV strain (EV S + G - R -);
- 1 c) a sensitive to streptomycin variant of the glycerol- and rhamnose-positive 1125 strain (1125 S-G+R+);
- 1 d) a highly resistant to streptomycin variant of the 1125 strain (1125 S+S+R+).

Before the test the cultures were purified by the repeated selection of isolated colonies. For obtaining mixed populations, equal volumes of bacterial suspensions, of approximately the same density, were combined and sown on agar plates. Two mixed cultures were prepared. They were made up of cells which differed from each other in three features: (EV S+G-R-) + (1125 S-G+R+) and (EV S-G-R-) + (1125 S+G+R+). For a control all the operations which were carried out with the mixed cultures were also carried out with the initial variants.

The test cultures were resown three times a week on Hottinger agar (26 reseedings were performed). After each reseeding the cultures were inoculated on common Hottinger agar, Hottinger agar with the addition of streptomycin up to a concentration of 1,000 units, Hottinger agar with 1 percent glycerol, Hottinger agar with the addition of glycerol and the

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antibiotic. For the stimulation of growth 1:10 hemolyzed blood was added to all the media and to the glycerol media for a control of the fermentation of trihydric alcohol an indicator (bromthymol blue) was added. The inoculation dose was selected with the calculation that the growth of 200 - 250 isolated colonies was obtained on one dish. The colonies which grew on glycerol media were selectively chosen for further study on Hiss' medium with glycerol and rhamnose. Primarily selected were colonies with a suspicion of a relation to glycerol which was not characteristic for the stated variant.

Reseeding of the mixed culture, made up of EV S+G-R- and 1125 S-G+R+, led to a quite rapid displacement of the streptomycin-resistant EV variant from the population; the ratio of the number of colonies grown on media with streptomycin to the number of colonies on agar without the antibiotic, which equaled 40% in the beginning of the test, dropped to 4% by the ninth reseeding (the control reseedings of EV S+G-R- did not bring forward the disappearance of resistance to the antibiotic). In connection with this, after the tenth passage we resorted to enrichment of the mixed culture with cells from the strain EV S+G-R-, and with a new drop in the content of streptomycin resistant cells we significantly increased the seeding doses during the seedings on streptomycin media. The overwhelming majority of colonies growing on glycerol-streptomycin agar did not ferment glycerol (825 colonies were examined). Only in one case (after 11 reseedings) among 51 colonies growing on glycerol-streptomycin agar was one of the features of glycerol fermentation detected. When studying this subculture it was established that it was highly resistant to streptomycin, and on Hiss' media it fermented glycerol (4--6 days) and rhamnose (6--9 days), that is, based on its properties it was very mindful of the variant 1125 S+G+R+. Seeding of the subculture into individual colonies showed that it was homogeneous based on the features of drug resistance and the fermentation of glycerol and rhamnose. The origin of the resulting variant could have been the result of the EV S+G-R- strain acquiring the capability to ferment glycerol and rhamnose, or due to the conversion of the 1125 S+G+R+ strain into a streptomycin resistant form. We suggest that a second possibility took place. In producing such a conclusion we take into consideration the relative ease with which the plague causative agent acquires a resistance to streptomycin and the comparative stability of the EV strain based on the characteristic of its relation to glycerol (with the control reseedings of the EV strain S+G-R- in 4977 and 4947 colonies, examined correspondingly on glycerol and glycerol-streptomycin agar, no signs of the fermentation of glycerol were detected). Further the problem arose, did the emergence of a new variant take place as a result of the interaction of the cells or did an abrupt increase take place in the sensitivity to the antibiotic in 1125 S+G+R+. Apparently the emergence of a new form was realized by the first method. In the appropriate control test (reseeding of strain 1125 S+G+R+) we didn't once observe the growth of colonies on media with the antibiotic, while on media without the antibiotic 12912 colonies on the whole were counted.

In the process of carrying out experiments which were not connected with the given work, we observed the sudden emergence of resistance in strain 1125 S-G+R+ only with seedings of large doses of the culture (on the order of hundreds of millions of cells) and in rare cases. We were able to obtain a result analogous to this one also during the carrying out of a repeated test. The biological mechanism of the phenomenon remains unclear.

In the tests with reseedings of the second mixed culture (EV S-G-R- and 1125 S+G+R+), just as in the previous case, the displacement of glycerol negative cells from the population took place. However, this process proceeded considerably slower. The ratio of the number of colonies on media with streptomycin to the number of colonies on media without the antibiotic, expressed by the share of glycerol positive cells in the population, grew from 44% in the first generations to 62% in the last passages. The overwhelming majority of colonies on glycerol-streptomycin agar fermented glycerol, however, in one subculture the absence of the ability to ferment this substance was detected. We cannot relate this fact as being due to the formation of a new form as a result of the interaction of the cells, since in the control (reseeding 1125 S+G+R+) we detected two subcultures with a sharply slowed down (up to 20 days) fermentation of glycerol.

The results of the tests speak for the fact that in a mixed population, consisting of glycerol positive (strain 1125) and glycerol negative (EV strain) cells, a slow displacement of the latter takes place, apparently due to their lower rate of growth. In such a population it is possible that there is a transfer of the feature of medicinal resistance from resistant bacteria to sensitivity. However, the frequency of this phenomenon is relatively small and does not exert a real influence on the rate and direction of population shifts.